2753-Pos Board B723
Aggregation of polyene antibiotics in aqueous solution. An MD study
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It has been proposed that the distinct action of polyene antibiotics on ergosterol or cholesterol containing membranes can be due to the insertion of the drug in the monomeric or aggregated form in the ergosterol containing membrane whereas in the cholesterol containing membrane only aggregates of the drug can insert into the membrane. Aggregation can occur in the aqueous solution previous to absorption onto the membrane. It is therefore interesting to study the aggregation of polyenes in aqueous solution. In this work a molecular dynamics study of the aggregation of Amphotericin B (AmB), and several derivatives having greater and smaller selectivity for ergosterol vs cholesterol membranes, where performed. The molecular mechanism for aggregation were studied. That is, if aggregation is driven by hydrophobic forces, hydrogen bond formation or dipole-dipole interactions. Validation of the potential constructed for the polyenes were tested against the known aggregation thresholds of AmB in water and dimethyl sulfoxide (DMSO), as well as a comparison with nuclear magnetic resonance (NMR) available for aggregation in DMSO.

2754-Pos Board B724
An Approach to Characterizing Single-Subunit Mutations in the Anthrax Protective Antigen Prepore and Pore
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Heptameric pores formed by the Protective Antigen (PA) moiety of anthrax toxin translocate the intracellular effector moieties of the toxin across the endosomal membrane to the cytosol. To elucidate the effects of selected mutations in PA, we devised a protocol for characterizing heptameric pore precursors (prepores) and pores containing an inhibitory mutation in only one subunit. We prepared monomeric PA containing two mutations: the test mutation and an innocuous Cys-replacement mutation (K563C) at an external site on the prepore. The introduced Cys was derivatized with a biotinylating reagent, and the mutated protein was allowed to cooligomerize with a 20-fold excess of wild-type PA. Finally, the biotinylated prepores were purified by avidin affinity chromatography and characterized in various assays. We used this protocol to examine mutations at D425 and F427, two residues where mutations are known to have strong inhibitory effects. The D425A mutation caused an inhibition by >104 of pore formation and a corresponding abrogation of transport activity. The F427A mutation caused ~100-fold inhibition of translocation across planar bilayers and smaller effects on pore formation and ligand affinity. These results show definitively that the protein transport activity of PA may be abrogated by altering a single residue in one subunit of the heptameric prepore, explaining the dominant-negative phenotype. The protocol described may be applied to the detailed characterization of various mutations in PA and other homooligomeric systems.

Epithelial Channels & Physiology

2755-Pos Board B725
Small G protein-induced trafficking of the Epithelial Na^+ channel
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The long term control of blood pressure involves Na^+ homeostasis through the precise regulation of the Epithelial Na^+ Channel (ENaC) in the aldosterone-sensitive distal nephron. ENaC activity is regulated, in part, by trafficking to the plasma membrane. Membrane levels of ENaC reflect constitutive delivery and regulated retrieval. To explore the action of small G proteins on the activity and trafficking of ENaC we utilized electrophysiological recordings of ENaC, biochemistry and fluorescence microscopy. RhoA increases ENaC activity by promoting channel trafficking to the plasma membrane. Direct visualization of ENaC movement near the plasma membrane with TIRF-FRAP revealed that RhoA accelerates ENaC trafficking toward the membrane. RhoA-facilitated movement of the channel was sensitive to disrupting the endomembrane system. Moreover, facilitating retrieval decreased ENaC activity but not trafficking toward the membrane. Rab11a and Rab3a are well-established as a participant in the regulation of recycling trafficking. Co-expression of Rab11a and Rab3a with ENaC results in a significant increase in channel activity. Biochemical and imaging methods demonstrate that Rab11a colocalized with ENaC and increased ENaC activity by affecting the plasma membrane levels of this channel. Rab11a increases ENaC activity in an additive manner with dominant-negative dynamin, which is a GTPase responsible for endocytosis. Brefeldin A, an inhibitor of intracellular protein translocation, blocked the stimulatory action of Rab11a on ENaC activity. This is consistent with a mechanism of increased trafficking toward the plasma membrane. We conclude that RhoA, likely through effects on the cytoskeleton, promotes ENaC trafficking to the plasma membrane to increase channel membrane levels and activity. Moreover, we hypothesize that ENaC channels, present on the apical plasma membrane, are being exchanged with channels from the intracellular (recycling) endosomes in a Rab11-dependent manner.
Supported by AHA and ASN.

2756-Pos Board B726
Quantitative Analysis Of DEG/ENaC Subunits Interaction
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Members of the DEG/ENaC protein family are voltage-independent, amiloride-sensitive cation channels. This family includes acid sensing ion channels (ASICs), which are involved in sensory function, modulation of learning and memory in the nervous system. Another branch of the DEG/ENaC family includes epithelial sodium channels (ENaCs) that are key players in sodium transport across epithelia. 'Classical' ENaCs are formed by three different subunits named α, β and γ. A forth subunit, δ, has been found in humans and other primates. It can form channels with ENaC β and γ but it is mainly expressed in non-epithelial tissues such as the nervous system. Several lines of evidence suggest that DEG/ENaC subunits may interact promiscuously to form heteromeric ion channels with diverse biophysical properties. Thus, δ could modulate αβγ channels or form channels with other DEG/ENaC subunits in tissues where α, β or γ are not expressed. ASICs have been proposed to interact with ENaC subunits to form heteromeric ion channels and also to directly modulate other ion channels such as BK. To assess the physiological relevance of these interactions, we need to: a) verify subunits co-expression in native tissues; b) quantify the efficiency of subunits interaction; c) biophysically characterize the different subunit assemblies. In this work we address some of these issues. We have quantified efficiency of subunits interaction by measuring membrane expression of fluorescently-tagged DEG/ENaC subunits in Xenopus oocytes. Our results show that δ is less efficient than α to form channels with β and γ subunits. Experiments to compare interaction efficiencies between other DEG/ENaC members and other ion channels are currently underway.

2757-Pos Board B727
Apical Methyl-β-cyclodextrin (mβCd) Treatment In A6 Renal Cells Does Not Affect Basolateral PGE_2-induced Cl^- Secretion , But Stimulates An Early Cl^- Peak Current, Activated By Hypotonic Shock
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We explore in this study whether cholesterol content of the apical membrane of distal kidney cells from *Xenopus laevis* (A6) affects the behavior of two types of anion channels, recently described by Bao et al. (AJP,2008,295,G234). Voltage clamp technique in an Ussing-type chamber, enabling the recording of short-circuit current, was applied on polarized monolayers of A6 cells, grown on permeable filter supports for 13 - 31 days. To create a gradient for Cl^- secretion, the apical solution was Cl^- and Na^+ free, whereas the basolateral solution contained normal NaCl. Twice basolateral addition of PGE_2 (1 μM) during 15 minutes with an interval of 60 minutes produced a first transient peak increase in I_{sc} of 7.25 ± 0.98 μAcm^{-2} (N=6), followed by a plateau of 2.77 ± 0.50 μAcm^{-2}. The second PGE_2 stimulation elicited a peak current of 6.63 ± 1.20 μAcm^{-2}. When the apical membrane was treated for at least 1h with 10 mM mβCD, PGE_2 evoked Cl^- secretion was not different from control. A hypotonic shock was induced twice with an interval of 60 minutes on the basolateral side by a sudden reduction of solution osmolality from 260 to 140 mOsm/kg H_2O. In control a transient increase in I_{sc} of 0.27 ±0.11 μAcm^{-2} (N=3) was observed during the second stimulation only. After 60 minutes treatment with 10 mM mβCD the hypotonic shock evoked an augmented transient I_{sc} of 1.82 ± 0.08 μAcm^{-2} (N=5). These results suggest that apical Cl^- channels in A6 cells, activated either by PGE2 or by cell swelling, are regulated differently by the membrane cholesterol content. This work was supported by the grants FWO Flanders G.0270.07 and BOF 03B0BF.

2758-Pos Board B728
Bile Acids Selectively Activate Iberiotoxin-sensitive Potassium Channels In Native Pancreatic Duct Cells
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Bile reflux into the pancreas is a common causes of acute pancreatitis. Our group has shown that luminal chenodeoxycholate (CDC) at low doses

(0.1mM) stimulated HCO_3^- secretion in intact pancreatic ducts (Venglovecz et al. Gut. 2008). This stimulatory effect of CDC on HCO_3^- secretion was caused by an IP_3-mediated elevation of intracellular calcium concentration and an increase in apical Cl^-/HCO_3^- exchange activity. The aim of this work was to investigate whether CDC could also regulate ion channels in native pancreatic cells. Using standard whole cell current recordings (KCl-rich pipette: NaCl-rich bath solutions) exposure of isolated pancreatic duct cells to CDC (0.1 mM) reversibly increased whole cell currents ~ 3-fold in 75 % of recordings (15/20 cells), and hyperpolarised membrane potential by ~ 18 mV. Resting and activated currents showed marked outward rectification, and were moderately voltage-dependent. CDC-induced currents were inhibited by external barium (5 mM, n=6), as well as by the selective high conductance K^+ channel blocker, iberiotoxin (100nM, n=7). However, they were not sensitive to TRAM34 (selective blocker of intermediate conductance K^+ channels) nor UCL 1684 (selective blocker of small conductance K^+ channels). Bile acid induced-activation was abolished when cytosolic Ca^{2+} buffering was increased with 5.0mM EGTA, and was only moderately reduced by removal of bath Ca^{2+}. Higher concentrations of CDC (>0.5 mM) were deleterious if exposed for prolonged periods. Together these results provide strong evidence that low doses of CDC selectively activate iberiotoxin-sensitive K^+ channels through an increase in cytosolic Ca^{2+}, primarily from internal stores. Activation of a K^+ conductance would hyperpolarise membrane potential and thereby increase the electrochemical driving force for HCO_3^- secretion through electrogenic apical anion exchangers. Supported by The Royal Society.

2759-Pos Board B729

MDCK Monolayers on Silicon Chips - The Epithelial Barrier Function on a Cellular Level

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The MDCK cell line is a general model system for epithelial cells. Insights about the resistance of the trans- and paracellular pathway on a cellular level come from electrical impedance spectroscopy [1]. We now aimed for a direct measurement of the cellular parameters with a variety of techniques to improve theoretical models of the epithelial cell line.(i) The cell-solid distance was determined by fluorescence interference contrast (FLIC) microscopy. (ii) Multi-Transistor-Arrays were used for measuring the thermal fluctuations of extracellular voltage that provide the electrical properties of the distributed resistor-capacitor system, in particular the sheet resistance of the cell-substrate junction. (iii) An alternating voltage is applied to an electrolyte/oxide/silicon capacitor below the cells and phase maps of the transmembrane voltage were measured in three dimensions with a voltage sensitive dye. These maps give resistances and capacitances of a three-dimensional equivalent circuit. In the case of imperfect confluency in comparison to confluent monolayers a surprising shift in distance to the substrate is found. The specific resistance in the junction beneath confluent layers of cells is enhanced. Time resolved measurements with different Na concentrations show an active regulation of the sheet resistance in the junction which can be blocked by inhibition of Na/K/Cl cotransporter. Trans- and paracellular resistances are accessible via transmembrane voltages. Therefore all important parameters on a cellular level can be determined.

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Intracellular Channels

2760-Pos Board B730

Electrophysiological Characterization of Mitochondrial Uncoupling Protein 1

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Uncoupling proteins (UCP1- UCP5) are integral transport proteins of the inner mitochondrial membrane (IMM). They mediate transmembrane ion leak, thus dissipating the electrochemical proton gradient across the IMM and uncoupling mitochondrial respiration and ATP synthesis. UCPs are involved in thermogenesis, reducing fat deposition, and attenuating reactive oxygen species production by mitochondria to protect the cell against oxidative damage and ageing. The mechanism of ion conductance of UCPs has long remained elusive due to the lack of direct methods for measuring ion currents produced by them. To resolve this problem, we applied the patch-clamp technique to the whole inner membrane of mitochondria from the brown adipose tissue to identify and characterize currents produced by the family's founding member, UCP1. In our experiments, both the cytoplasmic and matrix faces of the IMM were exposed to solutions that did not contain any ions normally permeable through ion channels or transporters, except for H^+ and OH^-. Under these conditions, we identified a current that showed all signature properties of UCP1: it was strongly potentiated by micromolar concentrations of unsaturated fatty acids, inhibited by removing endogenous membrane fatty acids with bovine serum albumin, and blocked by micromolar concentrations of purine nucleotides. Moreover, the current was absent in the IMM of the kidney COS-7 cells, in agreement with the fact that UCP1 is specifically expressed in the brown adipose tissue. The transport molecule mediating this current was activated by membrane depolarization and showed robust tail currents upon return to the negative potentials. In a symmetrical pH 5.0 solution the current amplitude was negligible as compared to a symmetrical pH 8.0 solution, suggesting that the current was carried by OH^- but not H^+. Our results indicate that UCP1 is a low-conductance OH^- channel weakly activated by membrane depolarization.

2761-Pos Board B731

TRIC-B Channels Is Critical For Physiological Functions Of Alveolar Epithelial Cells

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Trimeric intracellular cation (TRIC) channels act as counter-ion channels that function in synchronization with Ca^{2+} release from intracellular stores. TRIC channels have two subtypes (TRIC-A and -B) and form homo-trimers with a bullet-like structure. TRIC-B is present in most mammalian tissues, while TRIC-A is expressed in excitable tissues such as brain, heart and muscle. TRIC-A-knockout mice were viable and fertile, whereas TRIC-B-knockout mice showed neonatal lethality. Here we report on TRIC-B-knockout mice, which died within an hour after birth because of acute respiratory failure. TRIC-B-knockout mice exhibited severe cyanosis from appearance observation and respiratory acidosis from blood gas data. In light microscopy analysis, TRIC-B-knockout lung formed immature alveolar spaces and had many glycogen-rich cells. Ultrastructural analysis revealed reduction of type II alveolar epithelial cells (lamellar body positive cells), the number of lamellar body and area of lamellar body in TRIC-B-knockout type II cells. Fluorometric Ca^{2+} measurements showed that Ca^{2+} rise evoked by ATP was significantly reduced in the TRIC-B-knockout type II cells. Moreover, results from ionomycin and cyclopiazonic acid stimulation indicated Ca^{2+} contents in endoplasmic reticulum was significantly increase in TRIC-B-knockout type II cells.

These findings indicate that TRIC-B plays an essential role in glycogenolysis, pulmonary surfactant lipid metabolism, lamellar body biogenesis, maturation of type II cells and surfactant exocytosis. In addition, it is suggested that TRIC-B supported Ca^{2+} release from IP_3R in type II cells, because ATP-evoked Ca^{2+} response reduced in TRIC-B-knockout type II cells.

2762-Pos Board B732

Electrophysiological Comparison of TRIC-A and TRIC-B, Two Trimeric Intracellular Cation Channels Associated with Intracellular Ca2+−Stores

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TRIC membrane proteins have been shown to be present in the endo/sarcoplasmic reticulum (SR) and nuclear membranes of muscle cells and other tissues. TRIC-A has been demonstrated to be a cation-selective channel (Yazawa et al., Nature, 448, 78-82) but the function of TRIC-B has not been determined. We have therefore investigated whether TRIC-B exhibits similar functional properties to TRIC-A by reconstituting purified TRIC-B and TRIC-A into planar phosphatidylethanolamine lipid bilayers under voltage-clamp conditions. We find that TRIC-B also behaves as an ion-channel but with different properties from TRIC-A. Current-voltage relationships of TRIC-B in symmetrical 210 mM KCl or symmetrical 210 mM K-PIPES solutions revealed single-channel conductances of 59 ± 10 pS and 63 ± 5 pS (SD; n=5), respectively, suggesting that TRIC-B is cation selective. In comparison, the conductance of TRIC-A in symmetrical 210 mM KCl or symmetrical 210 mM K-PIPES solutions was 167 ± 26 pS and 158 ± 25 pS (SD; n=3), respectively. The K+ conductance observed with the TRIC-A channel is very similar to that reported by Miller and colleagues for the well known 'SR K+−selective channel' found in skeletal muscle (e.g. Coronado & Miller, J.Gen.Physiol. 79, 529-547). As for the SR K+−channel, we also find that TRIC-A is impermeable to Ca2+, is more open at positive potentials and exhibits a sub-conductance state approximately 50% of the full conductance level. The functional characteristics of TRIC-A and TRIC-B suggest that their localisation on intracellular Ca2+ stores may be vital for the counter-movement of ions to balance transient potential differences generated by Ca2+ movements across the SR membrane.

Supported by the British Heart Foundation, NIH and JSPS.